The Intersection of Neuroimaging and Genomics on Complex Traits and Perception

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THE INTERSECTION OF NEUROIMAGING AND GENOMICS ON COMPLEX TRAITS
AND PERCEPTION

by

HELENA C. YARDLEY

B.S., Florida State University, 2010

A thesis submitted to the
Faculty of the Graduate School of the
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Department of Integrative Physiology
2016
This thesis entitled:
The Intersection of Neuroimaging and Genomics
on Complex Traits and Perception

written by Helena C. Yardley,

has been approved for the Department of Integrative Physiology

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The final copy of this thesis has been examined by the signatories, and we find that both
the content and the form meet acceptable presentation standards of scholarly work in the
above mentioned discipline.
Thoughts, feelings and complex behavioral patterns are represented through neural patterns. These neural patterns have molecular and genetic underpinnings, but the connection between the two isn’t always clear. In this manuscript, we evaluate a possible neuroendophenotype for behavioral disinhibition in a sample collected from the Center on Antisocial Drug Dependence, who were selected based upon their degree of behavioral disinhibition. We obtained genome-wide data on the 1,901 participants, and generated a composite polygenic risk score for each of the 80 subjects from 1,876 single nucleotide polymorphisms shown to be associated with the behavioral disinhibition phenotype. We then use this PGRS to determine how genetic contributors drive activation in the brain during a risky and cautious decision making paradigm through functional MRI (fMRI).

To expand on this work, we focus on the full behavioral disinhibition GWAS. In this study, we collect data on behavioral measures encompassing novelty seeking, conduct disorder symptoms and substance dependence vulnerability. These measures collectively define the “BD” phenotype, and we inquire into whether adolescent BD is a
predictor for later life outcomes regarding BMI, drug vulnerability, continuing to higher education after high school, engagement in risky sexual behaviors and experience of depressive symptoms. The average time between the first assessment and subsequent follow-up was on average 9.2 years after initial BD measurements were obtained.

We then discuss the utility of using a meta-analytic approach to combine neuroimaging data across 256 studies on pain and touch perception. This database includes 4,665 subjects’ functional neuroimaging data from studies published from 1993 to 2015. Here, we use a Multi-Level Kernel Density Analysis inquiry into the most commonly activated brain regions during the presentation of pain, non-painful touch, and the relative differences between the two. We make additional comparisons on smaller sections of this database. Lastly, we will compare the MKDA maps of pain and touch to 7 resting-state default networks and then briefly discuss possibilities for the future with regards to complex neurophysiological and behavioral data and the importance of data sharing initiatives.
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Chapter I

The Challenges of Big Data: Tackling Complex Neurophysiological and Phenotypic Data with Different Analytical Techniques

“Big data” has been the buzzword in recent years, particularly with the boom of new methods and technologies that have been developed and fine-tuned in the past decade. Some of these developments include low-cost whole genome sequencing, advances in technology for neuroimaging, and a daily influx of new analytical and computational tools by which to analyze this abundance of data. As science and technology progress, we’re becoming even more aware of the complexities of human nature and behavior. Socrates said it best with:

“The more I learn, the less I know” – Socrates

One of the challenges in recent years regarding the quest for knowledge about human behavior focuses on the best approaches to wrangle these large and complex datasets. Here, we use different techniques to investigate complex neuropsychological phenotypes through large-scale meta-analysis, polygenic risk scoring of genome-wide data, and epidemiological approaches. Each approach has its benefits and unique methodological concerns. This manuscript will discuss ways that we can link each of these to further investigate the biological systems that may contribute to personality and behavior.
Mapping Brain and Genes to Mind

It is largely known that thoughts, feelings and complex behavioral patterns are represented through neural patterns \(^1\)–\(^3\). These neural patterns have molecular and genetic underpinnings, but the connection between the two isn’t always clear. Neural representations are beginning to show promise as an “endophenotype” by which to link possible genetic affiliates. Endophenotypes are heritable, neurophysiological, cognitive, or neurobiological traits, and are thought to be more closely related to the underlying pathophysiology of a larger behavioral phenotype than clinically observable constructs such as signs, symptoms, or diagnosis and therefore more proximal to the genetic substrate \(^4\).

In Chapter II, we evaluate a possible neuroendophenotype for behavioral disinhibition. We’ve recruited a sample (n = 80) of individuals from a larger sample (n = 1901) from the Center on Antisocial Drug Dependence (CADD), who are selected based upon their degree of behavioral disinhibition (BD), which is “the inability to inhibit behavior despite its social undesirability, and cascade of familial, educational, psychological, and legal consequences” \(^5\). We obtained genome-wide data on the 1,901 participants, and generated a composite polygenic risk score (PGRS) for each of the 80 subjects from 1,876 single nucleotide polymorphisms (SNPs) shown to be associated with the behavioral disinhibition phenotype (P < 0.01). We then use this PGRS to determine how genetic contributors drive activation in the brain during a risky and cautious decision making paradigm through functional MRI (fMRI).

To expand on this work, Chapter III focuses on the full behavioral disinhibition
GWAS sample (n = 1901). In this study, we collect data on behavioral measures encompassing novelty seeking, conduct disorder symptoms and substance dependence vulnerability (defined as lifetime number of symptoms from substances tried/lifetime number of substances tried; assessed across 10 substances)⁶. These measures collectively define the “BD” phenotype, and we inquire into whether adolescent BD is a predictor for later life outcomes regarding BMI, drug vulnerability, continuing to higher education after high school, engagement in risky sexual behaviors and experience of depressive symptoms. This allows us to collapse a series of behavioral indicators into one composite measure by which to predict subsequent outcomes at a later time period. The average time between the first assessment and subsequent follow-up was on average 9.2 years after initial BD measurements were obtained.

In Chapter IV, we will discuss the utility of using a meta-analytic approach to combine neuroimaging data across 256 studies on pain and touch perception. This database includes 4,665 subject’s functional neuroimaging data from studies published from 1993 to 2015. Here, we use a Multi-Level Kernel Density Analysis (MKDA) inquire into the most commonly activated brain regions during the presentation of pain, non-painful touch, and the relative differences between the two. Additionally, we compare pain in healthy subjects to pain in a heterogeneous clinical group suffering from chronic and recurring pain. We will also assess the commonalities in activation across different stimulus modalities, and the relative difference in activation between two of the most common stimulus modalities, thermal and mechanical pain stimuli. Lastly, we will compare the MKDA maps of pain and touch to 7 resting-state default networks.
developed by Yeo et al.\textsuperscript{7} to further understand the commonalities, and differences, between pain and touch, and other networks in the brain. In Chapter V, we will briefly discuss possibilities for the future with regards to complex neurophysiological and behavioral data and the importance of data sharing initiatives.
Chapter II

The Genetic and Neural Correlates of Behavioral Disinhibition.

Introduction

The ultimate goal within personality genetics is to find a biological substrate that accurately predicts or characterizes a particular behavioral phenotype. Genetics and neuroimaging are two emerging fields over the past 20 years that have been rapidly developing new techniques and analytical methods in order to get a closer look at the determinants of complex traits within the human population. Given the importance of genetics and environment in brain function, and the ability for neuroimaging to illuminate brain function, combining these two fields creates a unique opportunity for insight into the genetic determinants of personality and behavior. Here, we aim to shed light on the genetic and neural underpinnings of behavioral disinhibition (BD) through genome wide analysis of single nucleotide polymorphisms and functional neuroimaging, with hope of defining a neural endophenotype for decision making in high BD individuals.

BD is an underlying vulnerability and highly heritable trait\(^5\) characterized by a sustained behavioral disposition which encapsulates separate behavioral manifestations. BD-related behavior includes impulsive decisions, conduct disorder problems, substance dependence, and an inability to resist expressing inappropriate or restricted behavior\(^8\). For example, individuals may fail to refrain from unprotected sex, or to halt drug use as adverse consequences mount, whereas more inhibited persons may stop themselves. Recent research has shown high heritability of conduct disorders, in particular with antisocial and substance abuse problems, which are often correlated with BD\(^9\).
Exhibition of these risky behaviors are also associated with structural and functional abnormalities in prefrontal and limbic brain regions that mediate behavioral inhibition. For example, similar behavioral problems, lack of judgment, insight, and foresight are characteristic of “frontal disinhibition syndrome” caused by frontal lobe injuries. Behavior of high BD persons may resemble that syndrome.

Since roughly 84% of all genes are expressed in brain, it is reasonable to hypothesize that myriad polymorphisms in the genome might have a functional effect on how the brain processes information. We know that complex behavior doesn’t emerge from one genetic substrate, but that it is more likely polygenic, manifested from the cumulative and combined action of hundreds and thousands of tiny genetic effects from across the genome. These variations may be individually responsible for a cascade of molecular effects, resulting in multifaceted and complex phenotypes. Combining neuroimaging and genome-wide data comes with its methodological challenges. Comparing all SNPs individually against whole brain data leads to the typical issues we see with multiple comparisons, so for the purposes of this study, we used (PGRS) risk scores. These composite risk scores included all SNPs that were significantly associated with behavioral disinhibition from the GWAS at a threshold of p < 0.01. Previous studies have had success with the predictive accuracy of a polygenic approach particularly with behavior and externalizing disorders. We used this composite risk score to regress against the functional activity from our functional neuroimaging paradigm of risky/cautious decision paradigm to determine how an individuals genetic profile might influence the patterns of functional activity during decision making.
Methods

GWAS Participants

The participants from the previously published GWAS were collected from the Center on Antisocial Drug Dependence (CADD) sample. A detailed account of the participant inclusion criteria are provided in Derringer 2015. The GWAS sample included 1,901 unrelated adolescents that were over selected for traits exemplifying severe behavioral disinhibition. Participants were selected from both the community-representative (48.2 %) and high-risk samples (51.8 %). Average scores for BD were 0.19 (SD = 1.2, range = -1.9 to 5.0) for the community-representative participants and 2.76 (SD = 1.2, range = -0.3 to 6.7) for the high-risk participants. CADD GWAS participants had an average age of 16.5 (SD = 1.4, range = 13.0–19.9), 28.9 % were female, and 37.3 % of participants reported non-Caucasian ancestry (see Supplemental Derringer 2015 for complete demographic statistics).

MRI Inclusion Criteria

A subsample of the GWAS participants meeting our genetic and phenotypic requirements were selected for the neuroimaging and polygenic risk score portion of our study. 108 subjects were recruited for this portion of the study. The details of the final group demographics used for analysis are shown in table 1. A high BD group of males was selected from subjects whose combined BD scores were in the top two deciles for males. A high BD female group was similarly selected. An average BD Male group was selected from those whose combined BD scores ranged from +0.5 to -0.5 standard
deviation above or below the male mean combined BD score. An average BD Female group will be similarly selected. More detailed information about how the BD phenotype was defined is noted in *Phenotype Selection* below.

**MRI Exclusion Criteria**

MRI exclusion criteria included evidence of (1) marked claustrophobia; (2) current pregnancy (by test); (3) orthodontic braces; (4) color blindness; (5) other contraindications to MR scanning; including intracranial, intraorbital, or intraspinal metal, pacemakers, cochlear implants or other non-MR-compatible devices; (6) having a history of head injury with loss of consciousness for more than 15 minutes, neurological illness, or history of neurosurgical procedures. Additional exclusion criteria are (7) insufficient English to provide consent and to participate in interviews and testing; (8) subjects will not be obviously psychotic or intoxicated when providing consent; (9) IQ $\leq 80$ estimated from adolescent testing; (10) urine positive for THC, cocaine, methamphetamine, amphetamine, barbiturates, benzodiazepines, MDMA, methadone, other opioids, and PCP (AccuTest™); (11) and saliva positive for alcohol (AlcoScreen™).

22 subjects were excluded from the neuroimaging analysis due to unusable scan data. An additional 6 subjects were excluded from final analysis due to missing genotypic data, or genotypic data that did not pass quality check standards. Resulting high and average BD groups were age and gender balanced (High BD, $n = 40$ (20 males); Average BD, $n = 40$ (21 males)), as noted in table 1.
Study Subject Descriptives for age, sex and behavioral disinhibition

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age</th>
<th>Sex</th>
<th>BD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>High BD</td>
<td>40</td>
<td>28.28 ± 1.46</td>
<td>Female (n = 20) Male (n = 20)</td>
<td>1.09 ± 1.33</td>
</tr>
<tr>
<td>Average BD</td>
<td>40</td>
<td>28.12 ± 1.84</td>
<td>Female (n = 19) Male (n = 21)</td>
<td>-0.053 ± 1.04</td>
</tr>
</tbody>
</table>

Table 1: High BD and average BD groups were age and gender balanced

**Phenotype Description**

The BD phenotype for the GWAS was composed from measures of novelty seeking, conduct disorder symptoms and substance dependence vulnerability (defined as lifetime number of symptoms from substances tried/lifetime number of substances tried; assessed across 10 substances). A complete description of the BD phenotype construction can be found in Derringer 2015. Scores were normed to community-representative samples in CADD and applied to all CADD GWAS participants from both the community-representative (48.2 %) and high-risk samples (51.8 %). Average scores on the BD composite measure were 0.19 (SD = 1.2, range = -1.9 to 5.0) for the community-representative participants and 2.76 (SD = 1.2, range = -0.3 to 6.7) for the high-risk participants.

**Genotyping & Analysis**

All participants were genotyped on the Affymetrix 6.0 platform (Affymetrix, Inc., Santa Clara CA), with a total of 696,388 autosomal SNPs available for analysis after quality control. Population stratification was taken into consideration through multidimensional scaling in PLINK. Genome-wide analysis was conducted as a linear
regression of the additive effect of each SNP on BD in PLINK. All autosomal SNPs that passed basic quality controls were tested for association with BD, and 10 ancestry dimensions were included as covariates. Age and sex were accounted for in the estimation of the BD phenotype. The criterion for individual SNP significance was set at \( p < 5 \times 10^{-8} \).

**Discovery Sample for GWAS**

65 of the subjects from the neuroimaging sample and 15 of their monozygotic twins were included in the previously published GWAS. To ensure that the GWAS results are not inflated due to overlapping discovery and testing sets, we removed the 80 subjects from the 1901 GWAS participants, and re-ran the BD GWAS for SNPs associated with BD on the resulting 1821 participants. All the resulting SNPs from the new GWAS were pruned for linkage disequilibrium, SNPs correlated > 0.2 in the fMRI sample were removed, leaving a total of 187,751 SNPs. The p-values from these associations were then collected and binned to generate the cumulative polygenic risk score for our subsample of 83 subjects. 3 of these subjects were removed from final analysis due to unusable imaging data.

**Polygenic Risk Score**

A cumulative polygenic risk score was generated for each individual in the sample (n = 80). SNPs from the previously published BD GWAS were selected, rank ordered by their p-values, and binned accordingly. The total number of SNPs was 187,751. We created a total of 14 risk scores (e.g. \( p \leq .001, p \leq .005, p \leq .01, p \leq .05, p \leq \))
For each subject, we added the total number risk alleles for each SNP (0, 1 or 2). We then summed the risk alleles present for each SNP within the aforementioned bins, creating a composite score for each p-value threshold from the GWAS.

**Image Acquisition**

Scans were obtained on a 3T General Electric MRI scanner with a standard head coil. High resolution 3D SPGR anatomical scans (TE/TR/TI: 1.9/9/500 Flip:10 degree; FOV: 220X220 mm²; slices 124 each 1.7mm thick; scan time: 9:50;) were obtained for each subject. Functional scans were obtained using GRE-EPI (TE/TR/TI: 26/2000/70. Flip: 70 degree FOV: 220X220 mm²; 31 slices (5 of which had zshim applied) each 4 mm thick with 0 gap). Scan time for each session (total 3) was 10:59 with 1 min break and consisted of 329 scans;

**Behavioral fMRI Task**

After a mock training session, subjects played the Colorado Balloon Game (CBG), which is similar to Newman’s game 17. Participants were initially given $5 and could keep any additional earnings. In 90 “Decision Trials” subjects contemplated during a 4 second window whether to engage in a risky or a cautious decision. Then, during a 0.5 second window they made a left finger press response for a cautious decision with 1 cent earnings, or a right finger press response for a risky decision and either won 5 or lost 10 cents. The next 3.5 seconds informed the subject of the outcomes (with new dollar totals), followed by a 2-4 sec jittered fixation. The 90 Decision trials were paired with 90
“Directed trials” where the subjects just followed instructions to press right or left and were paid 2 cents for staying compliant. The directed trials provided a baseline to remove unwanted motor, visual and auditory cortex activations. We assessed brain activity during the 4-sec contemplation period, separately analyzing this period ending in cautious or risky responses.

Image Analysis

The raw dicom images were converted to Analyze format images after zshim correction (Du et al., 2007) for susceptibility artifact. The first seven scans from each session were removed to avoid saturation effects. Data preprocessing included motion correction, coregistration to the structural images, normalization to standard Montreal Neurological Institute (MNI) space, and smoothing with 6mm full-width-half-maximum Gaussian filter. For within-subject fMRI analyses we fitted preprocessed data with the general linear model (GLM) of Statistical Parametric Mapping (SPM8) software, filtering low frequency noise, correcting for temporal autocorrelation, and convolving with a single canonical HRF signal. A 128-s high pass filter removed signal drift and low-frequency fluctuation. The GLM model included these trial periods: decision, outcome (win or loss), and fixation. We generated single-subject contrast maps with SPM-8, analyzing brain-function differences in contrasts of interest (e.g. Risky Decision – Risky Directed & separately Cautious Decision – Cautious Directed) as fixed effects. We used SPM8’s multiple linear regression to regress each individual’s PGRS against the contrasts obtained for risky and cautious decision making, covarying for age (high BD age mean: 28.3 ±1.8; avg BD age mean: 28.1 ± 1.8), IQ (high BD IQ mean: 104.4 ± 10.6;
avg BD mean: 110.7 ± 9.6) and sex.

Results

Behavioral fMRI Task

Post-fMRI behavioral task analysis showed no significant differences in the outcomes for the decision making tasks in the MRI scanner (e.g. number of risky decisions, cautious decisions, wins, or losses) between groups for the CBG, which is consistent with a previous study with a similar sample group and design\textsuperscript{18}. There were trends with less risky decisions, more cautious decisions, less monetary loss and higher gains in the average female BD group, with the inverse being true for the high BD male group. The high BD group made an average of 53 risky decisions and 35 cautious decisions. The average BD group made an average 47 risky decisions and 40 cautious decisions. There were no significant gender differences within groups, risky and cautious decisions have been parsed by group and gender in table 2.
Behavioral Data for the Colorado Balloon Game

<table>
<thead>
<tr>
<th>Risky Decisions</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Female</td>
<td>49.40 ± 13.75</td>
</tr>
<tr>
<td>High Male</td>
<td>55.65 ± 18.00</td>
</tr>
<tr>
<td>Avg Female</td>
<td>44.42 ± 12.82</td>
</tr>
<tr>
<td>Avg Male</td>
<td>49.90 ± 13.98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cautious Decisions</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Female</td>
<td>38.60 ± 13.85</td>
</tr>
<tr>
<td>High Male</td>
<td>32.10 ± 17.67</td>
</tr>
<tr>
<td>Avg Female</td>
<td>43.26 ± 13.65</td>
</tr>
<tr>
<td>Avg Male</td>
<td>38.57 ± 13.94</td>
</tr>
</tbody>
</table>

Table 2: Behavioral data from the Colorado Balloon Game. No significant group or gender differences were observed.

Polygenic Risk Score

We generated a cumulative polygenic risk score for each of the 80 individuals, and compared these composite scores between the high and average BD groups. We ran one and two-tailed t-tests as well as a logistic regression to determine whether the polygenic risk scores were predictive of behavioral disinhibition, the results of which are summarized in table 3. We found a significant difference in the risk scores between the high and average BD groups when including SNPs that had a genome wide association of $p < 0.01$ and $p < .005$ with BD. As we included more SNPs in the risk score calculation, the composite score became less predictive of behavioral disinhibition. Below is a distribution of the polygenic risk scores for each of the significant GWAS bins (e.g. $p < 0.01$ and $p < 0.005$). The total number of SNPs included in each of these analyses were 3,576 and 1,876, respectively. Distributions for these SNP bins are shown in figures 1 and 2. We also ran a linear regression to determine whether the polygenic risk score predicted the severity of BD. Although our results were not significant in this model, there was a trend ($P = 0.0846$), as shown in figure 3.
Table 3: Group differences in composite genetic risk scores, binning the SNPs from the GWAS by increasing p-value ranges.
MRI Results

The results of the multiple linear regression yielded marked differences in activation during cautious and risky decision making tasks as a function of each participant's genetic risk score. Results are illustrated in figures 4 and 5. Subject’s polygenic risk scores were positively associated with activity in the right medial prefrontal cortex (rmPFC) and negatively associated with activity in striatal regions while

**Figure 2:** Polygenic risk scores for high (red) and average (grey) BD groups versus BD score. There was a trend in the relationship between the two ($p = 0.0846$).

**Figure 3:** Polygenic risk scores for high (red) and average (grey) BD groups versus BD score. There was a trend in the relationship between the two ($p = 0.0846$).

\textit{fMRI Results}

The results of the multiple linear regression yielded marked differences in activation during cautious and risky decision making tasks as a function of each participant’s genetic risk score. Results are illustrated in figures 4 and 5. Subject’s polygenic risk scores were positively associated with activity in the right medial prefrontal cortex (rmPFC) and negatively associated with activity in striatal regions while
deliberating on making a risky decision. There was a positive association of risk score with activity in the right dorsolateral prefrontal cortex (right dlPPFC) in the moments when subjects were deliberating on making cautious decisions. There were no negative associations of risk score with functional activity during cautious decision making.

<table>
<thead>
<tr>
<th>Risky decision making: positive</th>
<th>Risky decision making: negative</th>
</tr>
</thead>
</table>

![Brain images showing positive and negative associations with functional activity during risky decision making](image1)

**Figure 4**: Positive and negative associations with functional activity during the risky decision making portion of the Colorado Balloon Game. Positive associations during this period were seen in the mPFC, and negative associations were seen in striatal areas.

<table>
<thead>
<tr>
<th>Cautious decision making: positive</th>
</tr>
</thead>
</table>

![Brain images showing positive associations with functional activity during cautious decision making](image2)

**Figure 5**: Positive associations with functional activity during the cautious decision making portion of the Colorado Balloon Game. Positive associations during this period were seen in the dlPFC. There were no clusters of functional activity negatively associated with cautious decision making.
Discussion

Genome wide association studies look for relationships between the alleles of genetic markers and a phenotype of interest. In this study, we aimed to translate those results into a tool to determine how a genetic profile might drive neural activity and subsequent behavior and decision making. The results here are multi-faceted. Differences between average and high BD groups were identifiable through the generation of a risk score based on SNPs implicated in the BD GWAS. The risk scores generated from the p-value bins most highly associated with BD were most predictive of the phenotypic group, except for those SNPs with an association of p < 0.001. This provides evidence for the theory that common phenotypes may share common variants in the genome. We think that the lack of predictive power in the p < 0.001 bin may be due to a lack of the number of SNPs present in this bin. We expect that larger, better powered studies will likely continue fill out the association at this level. Conversely, as more SNPs were added into the calculation past an association of p < 0.01, the polygenic risk score became less predictive. It is reasonable to think that the SNPs most highly correlated with the BD phenotype would play a larger role in possibly driving the neural signatures associated with different decision processes. This process is likened to that of creating a neuropsychological evaluation, where symptoms more commonly associated with the disorder are used to create a composite score that is diagnostic. Here, we use genetic associations, instead of self-report, and the results are predictive of the phenotype. With increasing PGRS, we do see trends in the severity of the BD phenotype, as seen in figure 3.
The results of the linear regression of the PGRS against the imaging data were consistent from what we would expect. When deciding between making a risky and a cautious decision, and ultimately making a risky decision, subject’s functional activity was increased in the right mPFC and decreased in striatal regions as a function of their risk score. During the deliberation period where subject ultimately made a cautious decision, there was an increase in the dLPFC with increasing PGRS, and no discernable negative associations were seen during this time period as a function of PGRS. Since our group analyses included all the subjects, both the high and average BD groups, our results indicate the degree to which the PGRS drives this activity. There is a large body of literature surrounding the brain activity in these regions during decision making in healthy subjects and in those with high BD, conduct disorder and substance abuse. We know that the PFC and the striatum mediate each other during decision making within the context of risky behavior and reward, specifically that striatal dopamine signaling mediates top-down corticostriatal activity in order to control decision making. The prospect of reward consistently increases activity in the ventral striatum and the mPFC in healthy subjects, but the connectivity between these two may be altered in populations with high substance use and abuse. Koob and Volkow hypothesized that repeated intoxication-withdrawal cycles from drugs and alcohol may decrease dopaminergic responses to reward as a result of desensitization of reward circuits to non-drug rewards. These authors also suggest that extended drug use can lead to disruption of frontal activity in the anterior cingulate cortex, orbitofrontal cortex and dLPFC, leading to altered decision making and subsequent risk-taking behaviors. Other studies have shown a negative association between dLPFC and striatal activation for risk, during
decision making \textsuperscript{23}, which we have seen in this study as a function of PGRS. The dLPFC contributes to “higher order cognitive processes that regulate the selection among multiple competing responses and stimuli” \textsuperscript{24}. In our study, we saw an increase in the dLPFC during the cautious decision making period, which may suggest that more cognitive resources are being recruited in order to resist making a risky, and possibly detrimental decision.

The results of this study parallel conclusions that have been made in previous studies elucidating the neural mechanisms underlying decision making in healthy and risky populations, but this study has some limitations. One limitation we encounter here is that the PGRS was predictive in our sample, but more work needs to be done to replicate our findings in a larger sample of high BD subjects. This analysis will be part of our future directions to further understand what genetic contributors underlie the BD phenotype in a larger sample of 1901 CADD subjects. Another notable limitation with the study design is that our decision-making paradigms were divided into the deliberation period while making a risky or a cautious decision. Although this provides some clarity on decision making in the moment, these time periods were extracted post-hoc, depending upon the outcome after the decision-making period. So, it is possible that subjects may have “thought” they were making a risky decision, but the balloon didn’t pop, ultimately landing that data into the cautious category. Future analyses will include running the same analysis on the decision making period across both “cautious” and “risky” events, as our current analysis may not have adequately parsed out risky from cautious decision making processes.
Chapter III

Adolescent Behavioral Disinhibition and Later Life Health
and Behavioral Outcomes

So far, we have determined that a quantitative polygenic approach can be used to predict whether individuals might be more prone to developing the behavioral disinhibition (BD) phenotype from their genotypic profile, and that these polygenic contributions may drive neural activity associated with decision-making, and subsequent risky behaviors. Here, we expand upon Palmer et al.’s paper on BD predicting drug dependence and investigate further whether adolescent behavioral measures of BD can predict later health and behavior outcomes in early adulthood, specifically body mass index (BMI), depression, education, risky sexual behavior, as well as their composite drug vulnerability (DV) score at time 2. As mentioned previously, behavioral disinhibition is an underlying vulnerability and a highly heritable trait, encapsulating separate behavioral manifestations including impulsivity, conduct disorder, substance dependence, and other externalizing behaviors. Behavioral disinhibition has a high comorbidity rate with drug use and risky sexual behavior. Those diagnosed with conduct disorders at a young age often follow a path that lands them in the criminal justice system, failure to obtain higher education, and mental disorders.
Methods

Subjects

For the first time point, 1901 unrelated adolescents exhibiting high BD characteristics were selected from the Center on Antisocial Drug Dependence (CADD) sample. Half of the participants were drawn from high-risk populations that have a high incidence of involvement with the criminal justice system, substance abuse treatment, and attendance at special schools catering to this population. These participants were drawn from several previously published studies \(^{27-29}\). Participants had an average age of 16.5 (SD = 1.4, range = 13.0–19.9), 28.9 % were female, and 37.3 % of participants reported non-Caucasian ancestry. For the second time point, 1054 (~55% attrition) of the previously selected subjects were not able to be contacted, leaving a total of 847 subjects to be analyzed for later life measures. Age ranges for the different time periods are summarized in table 1.

<table>
<thead>
<tr>
<th>Wave 2</th>
<th>Wave 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>16.5 ± 1.4</td>
</tr>
<tr>
<td>Minimum age</td>
<td>13</td>
</tr>
<tr>
<td>Maximum age</td>
<td>19</td>
</tr>
<tr>
<td>N</td>
<td>1901</td>
</tr>
</tbody>
</table>

Table 1: Descriptives for wave 2 and wave 3 measures

BD Phenotype

At time 1, BD scores were derived using a composite measure of novelty seeking, conduct disorder symptoms and substance dependence vulnerability (lifetime number of symptoms from substances tried/lifetime number of substances tried; assessed across 10 substances). A complete description of the BD phenotype construction can be found in Derringer 2015 \(^6\). Principal component scores were normed to community-representative
samples in CADD and applied to all CADD participants from both the community-representative (48.2 %) and high-risk samples (51.8 %). Average scores on the BD composite measure were 0.19 (SD = 1.2, range = -1.9 to 5.0) for the community-representative participants and 2.76 (SD = 1.2, range = -0.3 to 6.7) for the high-risk participants.

**Time 2, Follow-up Measures**

At time 2, information about substance use, depression, highest education obtained and BMI were obtained from the Composite Diagnostic Interview Substance Abuse Module. Information about sexual behavior was obtained using a modified Risk Behavior Questionnaire developed for the CADD. BMI was calculated from height and weight measurements obtained at time 2. Educational attainment was derived from the question “What is the highest education degree or certificate you hold?”. Answers were rank-ordered ranging from “none” to “Doctorate: M.D., Ph.D., JD, etc.”, and then split into either 1) high-school degree or less, and 2) higher than high-school degree. Information on depression was obtained from the question “Have you experienced depression impairment in the last 12 months?”. Answers were categorized into yes/no. Risky sexual measures were pulled from two sources, one from the question “Since the AIDS epidemic began, have you sometimes had unprotected sex, that is without a condom, with someone who you thought could have the disease?”. Answers were recorded as yes/no. Additionally, a composite “life partner” score was derived from the question “Number of people (different partners) subject have had oral, vaginal, or anal sex with in your lifetime?”. Scores for this question were standardized and age corrected.
based on the mean of community participants. Dependence vulnerability (DV) was calculated by the lifetime number of symptoms from substances tried/lifetime number of substances tried, assessed across 10 substances. Average time between time 1 and time 2 assessments varied depending upon how long it took to locate the subject for the follow-up interview. The mean time between assessments was 9.2 years (SD = 2.7 years; range = 3.7-17.2 years). Note that total sample sizes for each model run in this study may vary due to missingness of various measures within the data.

Statistical Analysis

We constructed 3 linear models to investigate the relationship between BD scores at time 1 and subsequent BMI, DV and lifetime partner measures at time 2. Models included age, sex, and ancestry principal components as covariates. In addition to the 3 linear models, we also constructed 3 logistic regression models to further investigate educational attainment, depression and risky sexual behavior. Covariates for these models also included age, sex and ancestry principal components. As noted above, we created dichotomous variables for the outcome measures (Education: high-school education or less as “0”, and greater than high-school education as “1”; depression: no depressive impairment in the last year as “0” and depressive impairment in the last year as “1”; risky sexual behavior: unprotected sex with someone that could be infected with HIV as “1”, otherwise “0”).
Results

The results of the multiple linear regression models are presented in tables 2-4, and our logistic regression models are summarized in tables 5-7. All models use time 1 BD scores as a predictor for subsequent time 2 measures (i.e. BMI, drug vulnerability, risky sexual behavior, educational attainment, and depression). Age, sex, and ancestry principal component scores were included as covariates in all models. Ancestry principal components are not reported in the tables below. We did not find any associations of BD score with BMI, with all other factors being held equal. Age was associated with an increase in BMI in this model.

Table 2: Regression results for BD and BMI
n = 805, CADD Sample

<table>
<thead>
<tr>
<th>BMI Outcome</th>
<th>b</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>18.21</td>
<td>1.85</td>
<td>&lt; 2e-16 ***</td>
</tr>
<tr>
<td>BD</td>
<td>-0.038</td>
<td>0.13</td>
<td>0.76</td>
</tr>
<tr>
<td>Age</td>
<td>0.27</td>
<td>0.070</td>
<td>7.83E-05 ***</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.093</td>
<td>0.39</td>
<td>0.81</td>
</tr>
</tbody>
</table>

R-Squared = 0.03029

The analyses also controlled for genetic ancestry, which is not displayed in the above table.

As expected, adolescent BD did prove to be predictive of time 2 drug vulnerability measures. All other factors being equal, a one standard deviation increase in BD was associated with a 0.31 standard deviation increase in drug vulnerability measures in early adulthood (SE = 0.023), as shown in table 3.
Table 3: Regression results for BD and Drug Vulnerability  
\( n = 871, \text{CADD Sample} \)

<table>
<thead>
<tr>
<th>Drug Vulnerability Outcome</th>
<th>b</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.13</td>
<td>0.33</td>
<td>0.70</td>
</tr>
<tr>
<td>BD</td>
<td>0.31</td>
<td>0.023</td>
<td>&lt;2e-16 ***</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0036</td>
<td>0.012</td>
<td>0.77</td>
</tr>
<tr>
<td>Sex</td>
<td>0.068</td>
<td>0.071</td>
<td>0.34</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table  
R-squared = 0.1807

Adolescent BD was predictive of standardized life partner scores at time 2. All other factors being equal, a one standard deviation increase in BD score in adolescence was associated with a 0.24 standard deviation increase in standardized life partner score in early adulthood (SE = 0.017), as shown in table 4.

Table 4: Regression results for BD and Lifetime Sexual Partners  
\( n = 1034, \text{CADD Sample} \)

<table>
<thead>
<tr>
<th>Lifetime Partner Outcome</th>
<th>b</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.39</td>
<td>0.31</td>
<td>0.20</td>
</tr>
<tr>
<td>BD</td>
<td>0.24</td>
<td>0.017</td>
<td>&lt;2e-16 ***</td>
</tr>
<tr>
<td>Age</td>
<td>-0.024</td>
<td>0.018</td>
<td>0.16</td>
</tr>
<tr>
<td>Sex</td>
<td>0.071</td>
<td>0.059</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table  
R-squared = 0.1726

The results of the logistic regression models are shown in tables 5-7. All tables include odds ratios, 95% confidence intervals and P values. As expected, BD was predictive of obtaining higher than a high school education (OR = 0.61, 95% CI(0.55, 0.69). With all other factors being equal, for a one standard deviation increase in BD, there is a 44% lower odds of continuing education after high school (table 5).
Table 5: Results of Logistic Regression Predicting > High School Education
n = 847, CADD Sample

<table>
<thead>
<tr>
<th></th>
<th>Education Outcome</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>0.024</td>
<td>(0.0056, 0.10)</td>
<td>3.83E-07 ***</td>
</tr>
<tr>
<td>BD</td>
<td></td>
<td>0.61</td>
<td>(0.55, 0.69)</td>
<td>3.60E-16 ***</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>1.17</td>
<td>(1.11, 1.24)</td>
<td>2.98E-08 ***</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.62</td>
<td>(0.45, 0.85)</td>
<td>0.003329 **</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table.

In our logistic regression of depressive impairment (OR = 1.42, 95% CI(1.19, 1.68)), we found those with a one standard deviation increase in BD score in adolescence were 1.42 times more likely to have suffered depressive impairment in the previous year at time 2, with all other factors being held equal. We found that females in the sample also were 2.8 times more likely to have suffered depressive impairment in the previous year at time 2.

Table 6: Results of Logistic Regression Predicting Depression in the Previous Year
n = 847, CADD Sample

<table>
<thead>
<tr>
<th></th>
<th>Depression Outcome</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>0.069</td>
<td>(0.0037, 1.15)</td>
<td>0.064438 .</td>
</tr>
<tr>
<td>BD</td>
<td></td>
<td>1.42</td>
<td>(1.19, 1.68)</td>
<td>5.89E-05 ***</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>1.01</td>
<td>(0.90, 1.12)</td>
<td>0.87261</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.35</td>
<td>(0.19, 0.62)</td>
<td>0.000323 ***</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table.

Table 7 summarizes the second of our risky sexual behavior measures. All other factors being equal, a one standard deviation increase in BD score was associated with 1.35 times higher odds of having unprotected sex with a partner they suspected could be infected with HIV (OR = 1.35, 95% CI (1.09, 1.68)).
Table 7: Results of Logistic Regression Predicting Engagement in Risky Sexual Behavior
n = 846, CADD Sample

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.0054</td>
<td>(0.00017, 0.15)</td>
<td>0.0025</td>
</tr>
<tr>
<td>BD</td>
<td>1.35</td>
<td>(1.09, 1.68)</td>
<td>**</td>
</tr>
<tr>
<td>Age</td>
<td>1.05</td>
<td>(0.92, 1.19)</td>
<td>0.47</td>
</tr>
<tr>
<td>Sex</td>
<td>1.74</td>
<td>(0.80, 4.25)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table.

Discussion

Previous research has linked conduct disorder and behavioral disinhibition to a variety of detrimental life outcomes. We aimed to take a closer look. As expected, behavioral disinhibition scores from our CADD sample in adolescence were robustly predictive of risky sexual behavior, drug vulnerability, likelihood of obtaining higher education, and depressive symptoms later in life. Although the results of this study are illuminating, they may be limited in scope. The BD phenotype was derived from a series of measures novelty seeking, conduct disorder symptoms and substance dependence vulnerability. The youngest subjects at time 1 were 13, it is possible that they have not yet fully engaged in behaviors that might contribute to the BD score composition. Future investigations will involve computing a new score for individuals from measures obtained at a later age, but still within the adolescent age range. Another limitation of this study is the attrition rate from time 1 to time 2. We did find that BD was a strong predictor of missingness at time 2, as shown in table 8.
Table 8: Results of Logistic Regression Predicting Missingness at Time 2  
*n = 1901, CADD Sample*

<table>
<thead>
<tr>
<th></th>
<th>Missingness Outcome</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P Value</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.14</td>
<td>(0.038, 0.51)</td>
<td>0.003</td>
<td>**</td>
</tr>
<tr>
<td>BD</td>
<td>2.05</td>
<td>(1.90, 2.21)</td>
<td>&lt; 2e-16</td>
<td>***</td>
</tr>
<tr>
<td>Age</td>
<td>1.11</td>
<td>(1.02, 1.19)</td>
<td>0.007</td>
<td>**</td>
</tr>
<tr>
<td>Sex</td>
<td>0.68</td>
<td>(0.53, 0.87)</td>
<td>0.002</td>
<td>**</td>
</tr>
</tbody>
</table>

The analyses also controlled for genetic ancestry, which is not displayed in the above table.

For every one standard deviation increase in BD, subjects were 2.05 times more likely to be missing at time 2 (OR = 2.05, 95% CI (1.90, 2.21). This finding is important in that our results from the previous models are likely an underestimate of the impact of BD on later life outcomes, as a substantial amount of follow-up measures are missing as a function of BD. Further inquiries into this sample will involve obtaining follow-up data from the 1054 missing subjects at time 2.

In summary, we found that a composite score of BD is a reliable and accurate predictor of personality and behaviors exhibited in early adulthood. We hope to expand this study to additional subjects within the CADD sample, as well as continue to track their behavior and health outcomes in hopes of developing even more robust predictors within this sample.
Chapter IV

“Differential Modulation of Pain and Touch: An fMRI Meta-Analysis”

Introduction

Development of objective and statistically sound measures to characterize physical and emotional experiences in the body have been garnering more interest in recent years, and is becoming more attainable through the development of new and widely available analytical tools and publicly available datasets. Structural neuroimaging has been vetted and approved for use as a diagnostic tool for physical abnormalities, and has been shown to be predictive of a wide array of disorders including damage from head injury, degenerative disorders such as Alzheimer’s disease and to monitor other neurological disorders such as stroke, brain tumors and multiple sclerosis (National Institute of Neurological Disorders and Stroke). While structural imaging can be used to evaluate differences in brain morphology, functional magnetic resonance imaging (fMRI) can be used to identify common and divergent networks of activity in the brain during rest \(^ {33,34}\) and task performance \(^ {35}\). However, the use of fMRI as a diagnostic tool is not widespread, potentially because of the methodological challenges associated with functional neuroimaging, such as heterogeneous brains, patterns of activity, and the typically small sample sizes of fMRI studies. Upwards of 400,000 fMRI studies have been published since its inception around 1991, and every year we highlight more about how our experiences, thoughts, feelings, emotions and complex behavioral patterns are
represented through neural signatures\textsuperscript{1,2}. The physiologic and neural signatures of pain have been widely studied\textsuperscript{36}, and although pain perception is differentiated at the peripheral level\textsuperscript{37}, individual neuroimaging studies have been inconclusive about whether pain is associated with discriminable signatures at the neural level across stimulus modalities and stimulation sites. Large sample sizes are often difficult to obtain for these types of studies, making it difficult to pinpoint network involvement with reliability from one study to the next. To clarify how different networks of brain activity are involved in both pain and touch across both healthy and clinical populations, we have conducted a large meta-analysis of fMRI studies published on pain and/or touch from 1993 to 2015.

We aimed to both compare and differentiate common patterns of activation across pain and touch through a Multi-Level Kernel Density Analysis (MKDA)\textsuperscript{38}(software available from http://wagerlab.colorado.edu/tools). Our MKDA was conducted on 154 pain and 103 touch studies. We evaluated the following categories:

1) Common pain activations across all pain studies
2) Common activations across all touch studies
3) Relative differences in activations between pain and touch studies
4) Relative differences in pain activation between healthy and clinical populations
5) Common pain activations across stimulus modality
6) Relative differences in pain activation between thermal and mechanical stimuli
7) Comparisons of pain and touch activation to 7 resting-state networks

To avoid weighting studies in our analysis according to the number of coordinates reported, each contrast within a study is grouped into one contrast map, and these
individual contrast maps were then used as the unit of analysis and weighted by their sample size to create a more accurate weighting paradigm for the studies, representative of the number of subjects included in the maps, and not the number of peak activations reported. In this meta-analysis, we parsed out the commonalities in pain representation in the brain across stimulus modalities. We then analyzed the relative difference in the most commonly activated brain regions between painful and non-painful stimuli. We then characterized the differences between experimentally-induced pain within healthy samples with chronic and recurring pain in clinical samples. Aggregate maps for pain and touch were compared with the Buckner resting-state network maps \(^7\) including visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal and default networks to determine the similarity of pain and touch representations with other known networks. We then performed a series of pairwise support vector machine (SVM) classifications to understand further the common and divergent signatures of pain and touch stimuli.

**Methods**

We created a database of 256 studies encompassing neuroimaging paradigms that investigated the neural correlates of pain and touch. Studies included were published from 1993 to 2015, which included a total of 154 pain studies and 103 touch studies, resulting in 225 and 167 individual study contrasts, and 2785 and 1880 subjects, respectively. Each study contrast was coded based on a number of features (e.g. sample size, study contrast, stimulus modality, laterality of stimulus, location on the body, and whether the subjects were healthy recruitments, or part of a clinical sample).
<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Pain or Touch</th>
<th>Laterality</th>
<th>Body Part</th>
<th>Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>Pain</td>
<td>Left</td>
<td>Arm</td>
<td>Chemical</td>
</tr>
<tr>
<td>Patient</td>
<td>Touch</td>
<td>Right</td>
<td>Leg</td>
<td>Cold</td>
</tr>
<tr>
<td>Both</td>
<td>Both</td>
<td>Bilateral</td>
<td>Face</td>
<td>Heat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trunk</td>
<td>Mechanical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Visceral</td>
<td>Laser</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Electrical</td>
</tr>
</tbody>
</table>

Codes were entered manually and cross-checked by two team members. Disagreements with the coding were discussed, and settled between team members. Contrasts within studies were grouped into like categories, as shown in table 1. Activation foci and coordinates are reported in Montreal Neurologic Institute (MNI) standard anatomical space (or transformed from Talairach space). Foci are nested within study activation maps, maps of group comparisons between a pain or touch-related condition and a less intense or neutral comparison condition. We used the foci associated with study activation maps to predict each map’s associated pain or touch category. Studies were all peer-reviewed and were identified in journal databases (PubMed, Google Scholar, MEDLINE, and NeuroSynth) and in reference lists from other studies.

**Multi-Level Kernel Density Analysis**

Depending upon the contrast of interest, the database was parsed accordingly (e.g. healthy subjects + painful stimuli + touch stimuli + right-sided stimulation site). A Multi-Level Kernel Density Analysis (MKDA) was used to determine the spatial overlap of peak coordinates that consistently show increases in brain activity during the perception of pain and touch, relative to a less intense baseline, across previously published studies. The peak coordinates from each study contrast in the database were convolved with 10-
mm spheres to form binary indicator maps. These binary indicator maps serve as the unit of analysis for the MKDA in order to avoid over-weighting any one study reporting a higher number of peak coordinates within the database. Studies were weighted in the analysis by sample size. Point estimates of the proportion of unique study contrasts that reported activation for each contrast of interest were created, and MKDA maps were generated. Full details of the MKDA analysis methods can be found in (Lindquist 2016).

**Support Vector Machine (SVM) Analysis**

Next, we performed a ‘one-vs-one’ classification analysis of pain and touch, as well as subcategories of these conditions using a series of nonlinear SVM with parameters chosen a priori (slack parameter $C = 1$, radial basis function kernel with standard deviation = 10 mm) as implemented in the Spider machine learning toolbox for Matlab. To determine significance for the pairwise comparisons, a Monte Carlo simulation with 5000 iterations was performed. We then corrected for voxel-wise analyses, and family-wise error rate (FWER) in order to obtain results corrected for multiple comparisons across the whole brain at a significance of $P < 0.005$, $P < 0.01$ and $P < 0.05$. We used stratified 10-fold cross-validation to assess accuracy in classifying the stimulations associated with each study activation map, when ample study numbers were available. In each fold, approximately 90% of the maps were used to train the SVM algorithm (‘training set’), and the remaining 10% were used to assess classification accuracy (‘test set’). For each fold, we selected maps from the training set associated with each pairwise comparison of stimulation category (e.g., pain vs. touch, thermal pain vs. mechanical pain, etc.), and trained the SVM to predict those pairs.
<table>
<thead>
<tr>
<th>Type</th>
<th>Subject Group</th>
<th>Stimulus Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Chemical</td>
</tr>
<tr>
<td>Pain</td>
<td>182</td>
<td>7</td>
</tr>
<tr>
<td>Touch</td>
<td>141</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>431</td>
</tr>
</tbody>
</table>

Table 2: Contrast map counts for each type of study. Note that the modality counts are only including healthy subjects. Note that the “both” in group and “all” in modality do not equal the sum of the other categories, some studies in the database did not fit into one discrete category (e.g. pain that was thermal + mechanical; contrasts that included both healthy and clinical populations, etc.), but these studies are included in the overall maps of pain and touch.

**Results**

Our main interest was to determine what clusters of voxels were more commonly activated by pain and touch, respectively. This analysis revealed similar activation sites across pain and touch. Our results were consistent with previous meta-analyses showing consistent activation of the dorsal anterior cingulate cortex (dACC), bilateral insula, bilateral somatosensory cortex, bilateral striatum, and bilateral thalamus.

*Pain and Touch Across All Modalities*

As can be seen in figure 1, there is a significant overlap in the brain regions activated during painful and non-painful tactile stimulation. Both types of stimuli elicit activity within dorsal anterior cingulate cortex (dACC), bilateral insula, bilateral somatosensory cortex, bilateral striatum, and bilateral thalamus. This highlights the importance of using...
this form of analysis to clarify the relative differences between these two patterns of activation.

**Pain Versus Touch Across All Contrasts In Database**

*Figure 2: Pain vs Touch: 233 studies, 323 contrasts, 4665 subjects*

When comparing pain versus touch in healthy individuals, the map shown in figure 2 was able to discriminate between pain and touch contrast with 75% accuracy (78% pain, 70% touch). Within this map, pain-related contrasts show greater activity within bilateral insula, dACC, bilateral thalamus, striatum, the periaqueductal gray (PAG), and other brainstem areas. In contrast, tactile stimulation is associated with greater activity within bilateral somatosensory cortex.
Pain In Healthy Subjects Versus Patient Groups

Figure 3: Pain (healthy vs patients) 160 studies, 212 contrasts, 3037 subjects

Within this map (Figure 3), pain in healthy subjects was associated with greater activity within bilateral insula, bilateral thalamus, brainstem, caudate, and somatosensory cortex. In contrasts, pain within patients was associated with greater activity within dACC and prefrontal cortex. Accurate classification between pain in controls and patients could not be obtained, due to the unbalanced number of studies in the analysis (healthy: 182 contrasts; patient: 30 contrasts).
Pain Across Different Stimulus Modalities

These maps (figure 4) show that pain stimulation elicits similar activation across a range of modalities (e.g. chemical, cold, electrical, heat, laser and mechanical).

Unfortunately, there were not enough studies within each of the modalities to do a full cross-validated test of the differences between activation by modality within the cold, chemical and laser modalities, but the modality categories between thermal and mechanical had sufficient numbers. Figure 5 shows the relative differences between painful thermal and mechanical stimuli.
Thermal vs. Mechanical Pain

When comparing thermal pain stimulation and mechanical pain stimulation (figure 5), differential patterns of activation were seen in the periaqueductal gray (PAG), ventral tegmental area (VTA), insula and other brainstem areas for painful thermal stimulation, and more somatosensory representations for painful mechanical stimulation. The map was able to discriminate thermal from mechanical with 68% accuracy (67% thermal, 69% mechanical).

Pain and Touch: Relationship to Known Resting-State Cortical Networks

To further map pain and touch representations in the brain to other known networks, we compared the composite pain and touch MKDA maps to cortical parcellations of 7 known networks: visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal and the default network 7. Figure 6 illustrates these parcellations, which were created from resting-state data from 1000 subjects. Figure 7 depicts a positive correlation of pain experience to functional activity in the somatomotor, ventral attention and frontoparietal networks, and a negative correlation
with the visual limbic and default networks. A t-test on Fisher's r to Z transformed point-biserial correlations confirmed these relationships. There was no correlation of the pain MKDA map with the dorsal attention network. Correlations and p-values for these relationships are summarized in table 3.

Figure 8 illustrates that touch followed a similar pattern of association with the 7 networks defined by Yeo et al., though with a slightly more pronounced association with the dorsal attention network for mechanical stimuli. Overall, the touch MKDA map was positively correlated with the ventral attention, somatomotor and frontoparietal networks, and negatively correlated with limbic. However, our t-test on Fisher's r indicated that the only significant relationship of the touch MKDA map was a positive correlation with the ventral attention network, and a negative correlation with the limbic resting-state network. Summary stats are provided in table 5. Figure 7A and figure 8A illustrate the correlation of the individual stimulus modalities (e.g. thermal, mechanical, etc.) with the 7 networks. Figure 7B and figure 8B are the averages of all the stimulus modalities.
Table 3: Comparison of pain MKDA map to 7 resting-state networks

<table>
<thead>
<tr>
<th>Network</th>
<th>R</th>
<th>T</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>-0.054</td>
<td>-13.9581</td>
<td>0</td>
</tr>
<tr>
<td>Somatomotor</td>
<td>0.1643</td>
<td>6.501</td>
<td>0.0013</td>
</tr>
<tr>
<td>dAttention</td>
<td>0.023</td>
<td>2.0629</td>
<td>0.0941</td>
</tr>
<tr>
<td>vAttention</td>
<td>0.3567</td>
<td>12.713</td>
<td>0.0001</td>
</tr>
<tr>
<td>Limbic</td>
<td>-0.0465</td>
<td>-5.2217</td>
<td>0.0034</td>
</tr>
<tr>
<td>Frontoparietal</td>
<td>0.1174</td>
<td>6.9609</td>
<td>0.0009</td>
</tr>
<tr>
<td>Default</td>
<td>-0.0271</td>
<td>-2.8748</td>
<td>0.0348</td>
</tr>
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</table>

Table 4: Pearson’s R correlation of pain modalities to 7 resting-state networks

<table>
<thead>
<tr>
<th>Network</th>
<th>Chemical</th>
<th>Cold</th>
<th>Electrical</th>
<th>Heat</th>
<th>Laser</th>
<th>Mechanical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>-0.0508</td>
<td>-0.0403</td>
<td>-0.0612</td>
<td>-0.0551</td>
<td>-0.0491</td>
<td>-0.0671</td>
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<tr>
<td>Somatomotor</td>
<td>0.1685</td>
<td>0.0558</td>
<td>0.225</td>
<td>0.2075</td>
<td>0.1375</td>
<td>0.1917</td>
</tr>
<tr>
<td>dAttention</td>
<td>0.0129</td>
<td>-0.0025</td>
<td>0.0042</td>
<td>0.0088</td>
<td>0.0631</td>
<td>0.0513</td>
</tr>
<tr>
<td>vAttention</td>
<td>0.3243</td>
<td>0.2564</td>
<td>0.3736</td>
<td>0.4399</td>
<td>0.3495</td>
<td>0.3968</td>
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<tr>
<td>Limbic</td>
<td>-0.028</td>
<td>-0.0215</td>
<td>-0.0579</td>
<td>-0.0693</td>
<td>-0.0323</td>
<td>-0.0703</td>
</tr>
<tr>
<td>Frontoparietal</td>
<td>0.1031</td>
<td>0.16</td>
<td>0.0902</td>
<td>0.1155</td>
<td>0.0649</td>
<td>0.1709</td>
</tr>
<tr>
<td>Default</td>
<td>-0.0396</td>
<td>-0.0052</td>
<td>-0.0259</td>
<td>-0.0238</td>
<td>-0.0651</td>
<td>-0.0032</td>
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</tbody>
</table>
Table 5: Comparison of touch MKDA map to 7 resting-state networks

<table>
<thead>
<tr>
<th>Network</th>
<th>R</th>
<th>T</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>-0.0323</td>
<td>-2.4647</td>
<td>0.0569</td>
</tr>
<tr>
<td>Somatomotor</td>
<td>0.1215</td>
<td>2.4055</td>
<td>0.0612</td>
</tr>
<tr>
<td>dAttention</td>
<td>0.0475</td>
<td>1.172</td>
<td>0.294</td>
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<tr>
<td>vAttention</td>
<td>0.2311</td>
<td>10.1435</td>
<td>0.0002</td>
</tr>
<tr>
<td>Limbic</td>
<td>-0.034</td>
<td>-2.9197</td>
<td>0.033</td>
</tr>
<tr>
<td>Frontoparietal</td>
<td>0.0893</td>
<td>2.3592</td>
<td>0.0648</td>
</tr>
<tr>
<td>Default</td>
<td>-0.011</td>
<td>-0.7638</td>
<td>0.4795</td>
</tr>
</tbody>
</table>

Table 6: Pearson’s R correlations of pain modalities to 7 resting-state networks

<table>
<thead>
<tr>
<th>Touch Stimulus Modality</th>
<th>Chemical</th>
<th>Cold</th>
<th>Electrical</th>
<th>Heat</th>
<th>Laser</th>
<th>Mechanical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>-0.0219</td>
<td>-0.0645</td>
<td>-0.0596</td>
<td>0.0241</td>
<td>-0.0414</td>
<td>-0.0305</td>
</tr>
<tr>
<td>Somatomotor</td>
<td>0.0107</td>
<td>0.0049</td>
<td>0.2821</td>
<td>0.0982</td>
<td>0.069</td>
<td>0.2639</td>
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<tr>
<td>dAttention</td>
<td>-0.0145</td>
<td>-0.0096</td>
<td>0.0275</td>
<td>0.0653</td>
<td>-0.0222</td>
<td>0.2383</td>
</tr>
<tr>
<td>vAttention</td>
<td>0.2226</td>
<td>0.2811</td>
<td>0.1597</td>
<td>0.1782</td>
<td>0.2541</td>
<td>0.291</td>
</tr>
<tr>
<td>Limbic</td>
<td>-0.0155</td>
<td>-0.0425</td>
<td>0.0066</td>
<td>-0.047</td>
<td>-0.0292</td>
<td>-0.0766</td>
</tr>
<tr>
<td>Frontoparietal</td>
<td>-0.0055</td>
<td>0.2039</td>
<td>-0.01</td>
<td>0.1584</td>
<td>0.0395</td>
<td>0.1494</td>
</tr>
<tr>
<td>Default</td>
<td>-0.0167</td>
<td>0.023</td>
<td>-0.0265</td>
<td>0.0402</td>
<td>-0.0353</td>
<td>-0.0505</td>
</tr>
</tbody>
</table>
Discussion

In this meta-analysis, we found that the most commonly activated areas across modalities are within the ACC, S1, S2, insula, and the thalamus. This is consistent with previous meta-analyses on pain\textsuperscript{40,42,44,45}. We were able to discriminate with 75% accuracy between pain and touch across all modalities. Non-painful touch showed more activity in bilateral somatosensory cortex, whereas painful stimuli elicited activity in the other aforementioned areas.

Our comparison of pain in healthy subjects versus a heterogeneous group of clinical subjects suffering from different chronic and recurring pain showed interesting results. Markedly more activity was elicited in the PFC for clinical subjects. This finding is consistent with previous studies\textsuperscript{46–49} of chronic pain. A possible theory surrounding this finding is that the PFC is involved in reducing the affective symptoms of pain through top-down control\textsuperscript{50}, and that chronic pain may be represented through networks most commonly associated with emotional circuitry\textsuperscript{51}.

The MKDA maps showing the most common activations across different stimulus types appear mostly uniform, however, our contrast map between mechanical and thermal stimuli did show some differences. Mechanical stimuli tended to be represented more in the somatosensory cortex whereas thermal stimuli was localized in the PAG, VTA, insula and other brainstem areas. The characterization of thermal stimuli in the PAG and VTA is interesting in light of recent research by Charles Raison’s group, where
they’ve found that heating the body peripherally stimulates serotonin nuclei in these regions \(^5\).

In our comparisons with the available 7-network maps, we found that both pain had a significant positive association with the ventral attention, somatomotor and frontoparietal networks, a negative association with the visual, limbic and default networks, and no association with the dorsal attention network \(^7\). Our touch MKDA map had a significant positive association with the ventral attention network, and a negative association with the limbic network. No other associations were found here. This suggests that the experience of pain and touch might be related to the functioning of not only basic stimulation sensation networks, but also attention networks involved in orienting awareness to different situations in the external environment.

Individual neuroimaging studies tend to show unique representations of different painful and non-painful stimuli. The MKDA maps of pain across modalities look very similar, whereas our contrast maps showed some marked differences in how pain and touch are represented, respective of each other. However, just as the smaller studies that contributed to the meta-analysis may be prone to false positives and nefarious findings, we must also view the results of this meta-analysis cautiously. We know that data from neuroimaging studies with small sample sizes can lead to noisy and unreliable conclusions. In this analysis, we have attempted to overcome some of the pitfalls associated with data derived from small studies by compiling data from hundreds of previously published studies, however, this approach comes with it’s own methodological
concerns. It is possible that our results are biased due to the relative contributions of the studies to each map. For example, an imbalance in the numbers of contrasts (i.e. pain in patients (n=30) versus healthy (n = 182)) can bias the relative differences. In the future, we plan to re-run some of the above analyses with randomly selected, balanced groups. In addition to an imbalance of overall groups (i.e. patient vs. healthy), there may be imbalances embedded within the gross contrasts; within a right vs. left analysis, there might be more right arm stimulations, whereas there are more left leg stimulations. These imbalances may weight one side of the contrast preferentially, or lead to artifacts. In order to parse this out, contrasts would have to be equally balanced between group, laterality, stimulation type and location of stimulation. Unfortunately, this reduces the potential pool of studies with which to analyze each comparison down to numbers too small for our approach. In addition, these maps are mostly qualitative, which leaves room for interpretation. More comparative quantitative analyses need to be done in order to further parse out what differences, and commonalities, may underlie sensory perception. However, meta-analysis does provide a platform by which to bypass many of the issues that arise with smaller, individual studies, including small sample sizes, variability within and across studies, and scanner-to-scanner variability, in order to find consistency across all of these variations.
Both the fields of neuroimaging and genetics have been in their infancy over the last two decades, and it is only recently that we’ve seen a boom in new technologies (e.g. high-throughput sequencing), more elaborate analysis techniques, and multi-site studies allowing for faster accumulation of quality data. We have only seen the tip of the iceberg of imaging genomics, and many groups are developing more innovative techniques by which to link the two fields. A range of statistical methods have been used to probe associations between SNPs and functional neuroimaging data, including linear regression, parallel independent component analysis, multivariate analysis and a number of new statistical methods that pop up yearly. In this manuscript, we have discussed in depth a few of the ways in which complex neuropsychological data can be approached. In previous years, the candidate gene approach seemed to have great promise in understanding the genetic underpinnings of behavior, but ultimately provided little evidence for clear connections between the two. With the invent of high-throughput genome sequencing, we have been able to explore the combined association of individual loci on complex traits. Of course, these individual effects are small, and comparing the association each individual SNP to a phenotype of interest would likely prove futile, however, we can combine these individual contributors into an aggregate score (PGRS) that can be used as a metric for phenotype expression. In our study of behavioral disinhibition, we found that the PGRS generated from a previously published GWAS was
predictive of the BD phenotype, however, these results need to be replicated in a larger, unrelated sample.

We’ve also discussed the importance of collecting longitudinal behavioral and health-related data across the lifespan. In our analysis of a subset of the CADD sample, we were able to illustrate not only that a PGRS is predictive of BD in adolescence, but also that BD scores in adolescence are predictive of behaviors and health-related outcomes in early adulthood. Large-scale, longitudinal data provides a unique opportunity to assess not only behavioral and genetic correlates at a given time point, but the persistence of these behaviors throughout the lifetime. This kind of longitudinal work is important for identifying behaviors in adolescence that may predispose individuals to developing later substance use disorders, ending up in the criminal justice system, suffering from depression, or failing to obtain higher education. There are a number of current longitudinal data initiatives that provide open-access data, including the Genetics of Antisocial Drug Dependence (GADD, N = 111 families), the Colorado Longitudinal Twin Study, The National Longitudinal Study of Adolescent to Adult Health (Add Health), and many more. Continuation of these studies and collection of large-scale health and life outcomes is essential to understanding how early life behaviors affect later life outcomes.

Finally, we discussed the utility of compiling large neuroimaging datasets and investigating them from a meta-analytic approach. Neuroimaging studies have been contributing exponentially to the literature about the function of the brain. As these
studies accumulate, the integration of the individual findings across studies becomes increasingly more difficult to approach. Individual studies are often underpowered, and encompass substantial variability in the acquired data, in addition to variability from scanner to scanner across study sites. It is approximated that about 45 subjects are needed in order to achieve 80% power using Bonferroni correction on the whole brain. Smaller sample sizes can lead to inordinate false positive rates. This leads to inconsistency when attempting to delineate the most common activations in the brain across similar manipulations. Meta-analysis provides a unique opportunity to understand more deeply the commonalities of activations across studies, study sites, and experimental manipulations. In our meta-analysis, we were able to find the consistency of activations in response to painful and non-painful stimuli across upwards of 4500 scans. In addition, we were able to take these aggregate maps and find the relative, reliable differences in activation sites between each of these tactile manipulations through MDKA and machine learning. As future neuroimaging studies accumulate in the literature, the benefit of updating these meta-analyses will continue to grow. Neurosynth is an automated database that synthesizes human functional neuroimaging data from published studies, and can be used as a starting point for determining brain regions most commonly implicated in any given manipulation, though, it is not a substitute for carefully screening published data for nuances in different manipulations across studies.

In the future, the use of large-scale data integration, large-scale multi-site studies, and data sharing initiatives will help to give us a clearer picture of how we can map brain and genetics to mind, particularly with the weekly additions of new statistical tools and
machine learning packages. Integration of new sources of biological, molecular, and epidemiological data as well as collaborative efforts across study sites world-wide will open new doors for imaging and genomics in translational science that will further inform us on how complex neuropsychological data can be extracted from big data.
REFERENCES


